

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
30 June 2005 (30.06.2005)

PCT

(10) International Publication Number
WO 2005/059157 A3

(51) International Patent Classification:
C12P 21/06 (2006.01) *C07H 21/04* (2006.01)

(21) International Application Number:
PCT/US2004/041569

(22) International Filing Date:
10 December 2004 (10.12.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/529,407 11 December 2003 (11.12.2003) US
60/532,506 26 December 2003 (26.12.2003) US

(71) Applicant (for all designated States except US): **THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY** [US/US]; 1705 El Camino Real, Palo Alto, CA 94306-1106 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BLAU, Helen, M.** [US/US]; 580 Cotton Street, Menlo Park, CA 94025 (US). **SEN, George** [US/US]; 334 Olmstead Road, Apt. 220, Stanford, CA 94305 (US). **MYERS, Jason** [US/US]; 635 Cambridge Avenue, Menlo Park, CA 94025 (US). **WEHRMAN, Tom** [US/US]; 269 Campus Drive, Stanford, CA 94305 (US).

(74) Agents: **FIELD, Bret, E.** et al.; Bozicevic, Field & Francis LLP, 1900 University Avenue, Suite 200, East Palo Alto, CA 94303 (US).

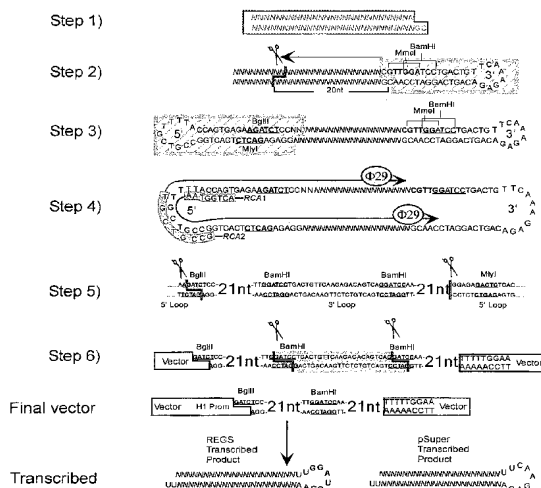
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— with international search report

[Continued on next page]

(54) Title: METHODS AND COMPOSITIONS FOR USE IN PREPARING HAIRPIN RNAs



(57) Abstract: Methods and compositions for producing hRNA, e.g., shRNA, expression modules for specific target nucleic acids are provided. In the subject methods, an initial nucleic acid, e.g., dsDNA, synthetic DNA, etc., corresponding to the target nucleic acid of interest is converted to an intermediate nucleic acid. The resultant intermediate nucleic acid, following an optional size modification step, is then converted to a linear dsDNA that includes at least one copy of the hRNA expression module of interest, or a precursor (i.e., pro-shRNA expression module) thereof, where in certain embodiments conversion may include amplification. Also provided are reagents, systems and kits for use in practicing the subject methods. The subject methods and compositions find use in a variety of different applications.

WO 2005/059157 A3



— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(88) Date of publication of the international search report:

27 April 2006